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Phenolics and antioxidant activity of American and hybrid hazelnuts

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Abstract
Hazelnut leaves, involucres, shells and nutmeats were tested for levels of phenolics and antioxidant activity. Leaves were found to be the highest in phenolics, while involucres and shells were similar and nutmeats had the lowest. All showed strong antioxidant activity, with leaves and involucres having the highest, shells having next and nutmeats having the least. Overall, more research is needed to characterize these phenolics and determine whether they have potential as a natural food additive for use as an antioxidant.

Keywords: bitter, filbert, tannin, catechin

1. Introduction
Thomas Jefferson, the third United States president and noted horticulturalist, once wrote"…the greatest service which can be rendered any country is to add an useful plant to its culture; especially, a bread grain; next in value to bread is oil." [1] Selection of oilseed crops has a long history and has the potential to have significant economic returns [2]. European hazelnuts (*Corylus avellana* L.) have been subjected to informal selection efforts for many hundreds of years, and to formal efforts, for more than 50 years. American hazelnuts (*Corylus americana* Marshall), by comparison, have had a relatively insignificant history of selection and such efforts have been predominantly for identification of disease resistant parents for use in breeding programs for European hazelnut. American hazelnut is native to the upper Midwest of the United States and is a common shrub in forested areas of that region. Development of American hazelnut as an oilseed crop is underway with exciting potential.
An American hazelnut selection effort was started in Wisconsin in 2008 [3]. As a part of that project, they compared American hazelnut to a European processing variety (Tonda di Giffoni) and found that some selections of American hazelnut had higher levels of bitterness [4]. Bitterness is a defect that is commonly selected against for many crops. Indeed, at least a portion of the history of plant selection can be viewed through the lens of reducing bitter compounds within wild crop plant progenitors [5]. However, while these bitter compounds may reduce the palatability of some crop plants, they may contribute significantly to the food's health promoting properties. Hazelnut skins have significant levels of phenolic compounds (many of which are perceived as either bitter or astringent) and those compounds may be of nutritional value [6, 7]. Certainly, European hazelnuts are one of the best sources of polyphenolics in the human diet [8].
Polyphenolics often demonstrate antioxidant potential and these antioxidants may have positive human health impacts [9]. Beyond potential health promoting characteristics, antioxidants also have industrial value as food preservatives. The antioxidant value of European hazelnuts and waste products has already been tested and may have food science applications [6, 10]. While it is known that European hazelnuts have this potential, the potential of American hazelnuts as well as interspecific hazelnut hybrids, is not known. We hypothesize that American hazelnuts and its interspecific hybrids could have higher levels of phenolics, because selection has not been done against bitterness and astringency as has happened both formally and informally with European hazelnut. The leaves, stems and involucres are of particular interest, as they are currently a waste product and could become a valuable co-product, if they were shown to have antioxidant potential.
The objective of this exploratory study is to determine the levels of phenolics within a subset of our wild selections of American hazelnut and interspecific hazelnut hybrids and then to test these extracts for antioxidant potential.

2. Materials and Methods

To allow comparison between our results and those found for *C. avellana*, protocols of Alasalvar *et al*. and Shahidi *et al*. were followed for phenolic extraction and testing of antioxidant potential (with minor alterations).[10,11]Samples of leaves, nutmeats, shells and involucres were collected in September 2015 from five American hazelnuts and eight interspecific hybrid hazelnuts. The samples were frozen at -80 ^{O}C until used.

2.1 Processing

Samples were ground in a Wiley Mill. Fats were removed using hexane, 1:10 weight to volume, three times for five minutes each. Samples were then air-dried (12 hours) and stored in -20 ^{O}C.

2.2 Extracting

The solvent used was 80:20 ethanol/water (v/v).Extraction was at 1:5 ratio (v/v) at 80 °C under reflux conditions for 30 minutes. Samples were extracted and centrifuged (Thermo Scientific Sorvall ST40R; 4000g, 15 minutes) a total of three times, with supernatant liquids removed each time and combined with previous aliquots. Solvent was evaporated at 40°C under vacuum. Samples were then freeze dried (LABCONCO Freezone 4.5; -48 °C/0.046 mbar/72 hours) to dryness and stored at -20 °C.

2.3 Total Phenolics

Following methods of Shahidi *et al*., methanol was used to dissolve extracts at a concentration of 1 mg/ml of nutmeat or 0.5 mg/ml of leaf, shell or involucre extract [11]. 0.5 ml of extract and 0.5 ml of Folin-Ciocalteu phenol reagent were mixed in centrifuge tubes. We added saturated sodium carbonate solution (1 ml).Volume was adjusted to 10 ml using DI water. Tubes were vortexed and followed by 45 minutes of resting at room temperature. Tubes were centrifuged (Thermo Scientific Sorvall ST40R; 4000g, 5 minutes).Absorbance was measured (Thermo Scientific Evolution 60S) at 725nm.Absorbance readings were compared to a standard curve (catechin).

2.4 Antioxidant Activity

Following slightly altered methods of Shahidi *et al*., 0.1 M phosphate buffered saline (PBS) was used to dissolve the extracts prepared in Section 2.2 [11]. For leaves and involucres, 5 ml of PBS was used. For nutmeats and shells, 1 ml of PBS was used. The solutions were filtered through a 0.45-µm membrane filter. Product was diluted to 200 ppm catechin equivalents (CE). This dilution was based on measured CE equivalents determined in Section 2.3.Next, 43mM hydrogen peroxide was prepared in 0.1 M PBS. We mixed 0.6 ml of the hydrogen peroxide solution into each tube. Tubes were allowed to react for 40 minutes at room temperature and then absorbance was measured (Thermo Scientific Evolution 60S) at 234 nm. Drop in absorbance was compared to a standard curve for hydrogen peroxide. Absorbance readings were corrected for background using samples without hydrogen peroxide.

2.5 Statistics

Results for wild versus hybrid plants were compared using standard errors due to unequal sample size between wild and hybrid plants. ANOVA was used to compare levels of phenolics and antioxidant activity for the different plant parts with plant considered a blocking factor (and wild versus hybrid plant ignored as a factor; MiniTab 17.2.1.0).

3. Results and Discussion

There were no significant differences between the American and hybrid hazelnut extracts for either total phenolics or antioxidant activity (Table 1). As both are at a relatively low level of domestication, this is not a surprise. Plant parts were significantly different in level of phenolics (F-stat=57.7, p<0.0001) and antioxidant activity (F-stat=201.4, P<0.0001). Leaves contained the highest levels of phenolics (225±12 mg CE/g), with involucres (159±12 mg CE/g) and shells (154±13 mg CE/g) being similar, and nuts being the lowest (21±2 mg CE/g). Involucres (proportion hydrogen peroxide consumed 0.97±0.01) and leaves (0.96±0.01) were similar for antioxidant activity, shells had significantly less antioxidant activity (0.80±0.01) and nutmeats (0.65±0.02) had the least antioxidant activity.

Shahidi *et al*. found European hazelnut leaves, involucres, shells and nutmeats to contain (means) 135, 127, 214 and13.7 mg CE/g, respectively [11]. Alasalvar *et al*. found European hazelnut involucres to contain 156 mg CE/g and nutmeats to contain 23 mg CE/g (when extracted by a method comparable to the one we used) [10]. Comparably, we found American and hybrid hazelnuts leaves, involucres, shells and nutmeats to contain (means) 225, 159, 154 and 21, respectively. Overall, the levels of phenolics are not largely different between our estimates for American hazelnuts and hybrids and the estimates of Shahidi *et al*. and Alsalvar *et al*. for European hazelnuts [10, 11]. However, American hazelnuts may contain higher levels of phenolics in their leaves; while their involucres and nutmeats may have similar levels of phenolics to European hazelnuts and their shells may have less phenolics when compared to European hazelnuts. Overall, the low levels of phenolics in the nutmeats is not surprising (as it is the part of the plant that is actually eaten).

Antioxidant activity, as measured by ability to consume hydrogen peroxide, was extremely similar to findings by Shahidi *et al* [11]. Our leaves, involucres and shells consumed 96, 97 and 80%, respectively. Shahidi *et al*. found 99, 97 and 99%, respectively, when using the same concentration of mg CE/g that we used [11]. Based on both levels of phenolics and antioxidant activity, European hazelnuts may have stronger phenolics in their shells. We found 65% hydrogen peroxide consumption for American hazelnuts while Shahidi *et al*. found 77% for European hazelnuts.[11]Because both their study and ours based antioxidant activity off of samples that were diluted to 200 ppm CE, similar levels of consumption of hydrogen peroxide between plant parts is not surprising. However, both the level of total phenolics as well as the strength of the antioxidant activity of those phenolics in the other plant parts is much higher than in the nutmeats themselves.

These results are quite exciting. The high levels of phenolics and their strong antioxidant potential, within parts which are otherwise considered waste products, may provide an opportunity if more research is done. This study was, of essence, exploratory in nature. Until this time, no one had compared American and hybrid hazelnut phenolics and antioxidant activity to European hazelnuts. For both crops, there appears to be a significant opportunity to develop potential natural human or livestock food additives for use as antioxidants. The obvious next step would be characterization of the phenolics (as has already been done for European hazelnuts) and determination of their activity in food/feeds.

Table 1: Total phenolics in American and hybrid hazelnut nutmeats, shells, leaves and involucres presented as catechin equivalents (CE) and antioxidant activity as measured by ability to consume hydrogen peroxide. Data is presented as means with standard error in parenthesis.

		Nutmeat	Shell	Leaf	Involucre
		mg CE/g			
Total Phenolics	Hybrid	18.7(3.0)	162.0(20.1)	234.9(22.1)	160.8(15.7)
	Wild	23.5(3.9)	140.8(11.1)	207.7(26.2)	155.7(18.6)
		Proportion hydrogen peroxide consumed			
Antioxidant activity	Hybrid	0.63(0.02)	0.81(0.01)	0.95(0.01)	0.97(0.01)
	Wild	0.67(0.03)	0.79(0.01)	0.98(0.01)	0.97(0.01)

4. Conclusions

Hazelnut residues (involucres, leaves and shells) have quite high levels of phenolics while nutmeats are somewhat lower. Overall, their phenolics demonstrate significant antioxidant capacity when tested with ability to consume hydrogen peroxide. There appears to be a potential, with further research and development, for these products to be used as antioxidants for commercial use.

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